RP-HPLC DETERMINATION OF B-CAROTENE FROM THREE MAIZE HYBRIDS

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The paper presents a method for determination of β-carotene concentration from three maize hybrids (NSSK 444, NSSK 640 and Lovrin 400) carotenoidic extracts using the reversed phase- high performance liquid chromatography (RP-HPLC). The analysed hybrids were cultivated at the Didactic Station of the Agricultural Science and Veterinary Medicine Banat's University, Timisoara. In view of a more complete carotenoidic pigments liberation from the raw material, it was used an improved carotenoids extraction technique. In this purpose, before of the carotenoidic compounds solvents extraction, the maize flour was moisten with distilled water and then treated with ethanol 96 % and let at rest 50 minutes for starch hydrolysis and advanced liberation of the carotenoids from plants cells. Carotenoidic pigments extraction was achieved with an organic solvents mixture of petroleum ether: acetone: ethanol 96% (6:3:1, v:v:v) to colorless. For the RP-HPLC analysis it was used an Agilent 1100 system equiped with a Zorbax SB-C18 column, 250 x 4,6 mm and particles size of 5 μm,UV/VIS detector with variable wavelenght and HPChemStation software. β-Carotene was identified in all the three maize hybrids. All the three analysed maize hybrids (NSSK 444, NSSK 640 and Lovrin 400) presents a very high β –carotene content, compared with the literature data concerning β–carotene content in other maize hybrids. The highest β –carotene content was founded in the NSSK 444 hybrid (670.30 μg/g), and the lowest in the Lovrin 400 hybrid (115.50 μg/g). In the NSSK 640 maize flour was founded a β –carotene content of 444.60 μg/g.

***Keywords**: β -carotene, maize, HPLC analysis.*

Maize (*Zea mays*), *Gramineae* family is, after wheat and barley, the most important grain in the world. Exists a lot of maize convarieties from which were obtained numerous hybrids with different physical-chemical characteristics, following especially the increase of maize nutritive value, for a superior utilization in human and animal food. In contrast with the most of the other cereals, maize

contains in the seeds (in endosperm) a great number of carotenoids: □- and □-carotene, □-zeacarotene, □- and □-cryptoxanthin, lutein, zeaxanthin, violaxanthin and others [1,4,11]. In the present are achieved researches for obtaining new maize forms (hybrids, varieties) with a high □-carotene content. This maize forms are generic named: „high beta carotene maize" (HBCM) [14] or „carotenoid-biofortified maize" [7,8].For quantitative determination of carotenoidic pigments and their metabolites, in the present, the most used method is reversed phase- high performance liquid chromatography (RP-HPLC) [2,3,6,9,10,12,13].

MATERIALS AND METHODS

Reagents, materials and apparatus

As raw material for the carotenoidic extracts obtaining were used the grains of three maize hybrids: *NSSK444*, *NSSK640* and *Lovrin 400* cultivated at the Didactic Station of the Agricultural Science and Veterinary Medicine Banat's University Timisoara. The extraction solvents: ethanol (96%), petroleum ether and acetone were purchased from Chimopar, Bucharest. Butylhydroxytoluene (BHT) used for carotenoids oxidative degradation prevention was from Merck company like the potassium hydroxide used for saponification process. The solvents for RP-HPLC analysis: acetonitrile and methanol were from Merck & Co., Inc., New Jersey, and the β-carotene standard proceeding from Sigma Chemical Company.

The laboratory apparatus used was: rotative evaporator model RV-05 basic 1-B (Shimadzu, Japan); centrifuge model Universal 32 R (Hettich, Germany);digital analytical balance model AW 320 (Shimadzu, Japan); HPLC system Agilent 1100 (Agilent, USA).

Obtaining of the carotenoidic extracts

For the carotenoidic extracts obtaining, the dried maize grains were grinded to a fine powder obtaining. The measured maize flour was moisten with distilled water, then treated with ethanol 96% and let at rest 50 minutes- for starch hydrolysis and advanced carotenoids liberation from cells. For carotenoids oxidative degradation prevention was aded BHT 0,1% (reported at the raw material). Carotenoidic pigments were extracted with a solvents mixture of: petroleum ether: acetone: ethanol 96% (6:3:1, v:v:v) [11,12]. The etheric extracts were concentrated under vacuum, at 35ºC, in an rotative evaporator and submitted to saponification with 40 ml KOH 20% ethanolic solution, for 16 hours at room temperature and in darkness. Carotenoids were then reextracted with petroleum ether in a separation funnel, washed for several times with NaCl saturated solution and then with distilled water until the complete removing of soaps and alkalies. The etheric extracts were treated with anhydrous Na_2SO_4 for dehydration, filtered and then concentrated under vacuum, at 35ºC, for complete removing of the ether. Samples were redissolved in petroleum ether and keeped at -20 ºC. From every maize hybrid were achieved three samples in the same conditions.

RP-HPLC analysis of carotenoidic extracts

The extracts obtained from the three maize hybrids were submitted to reverse phase high performance liquid chromatography (RP-HPLC) analysis in view of β-carotene concentration determination. The HPLC system Agilent 1100 was equiped with a Zorbax SB-C18 column, 250 x 4,6 mm and particles size of 5 µm. As mobile phase was used a mixture of acetonitrile: methanol (20: 80 v/v), with 1 ml/min eluent flow, a temperature of 30ºC and the wavelength of 450 nm. Were injected samples of 20 µl and for β-carotene concentration determination was used a standard curve obtained with pure β-carotene.

RESULTS AND DISCUSSION

Standard curve obtained with pure β-carotene conduct to the following calibration equation:

$$Area = 7.8 + 862.7 \cdot c\,; \quad r = 0.969$$

where *Area* represents the peak area expressed as mAU·min, *c* is the β-carotene concentration in g/100 ml, and *r* is the correlation coefficient.

The RP-HPLC superposed chromatograms obtained for standard β-carotene are presented in figure 1.

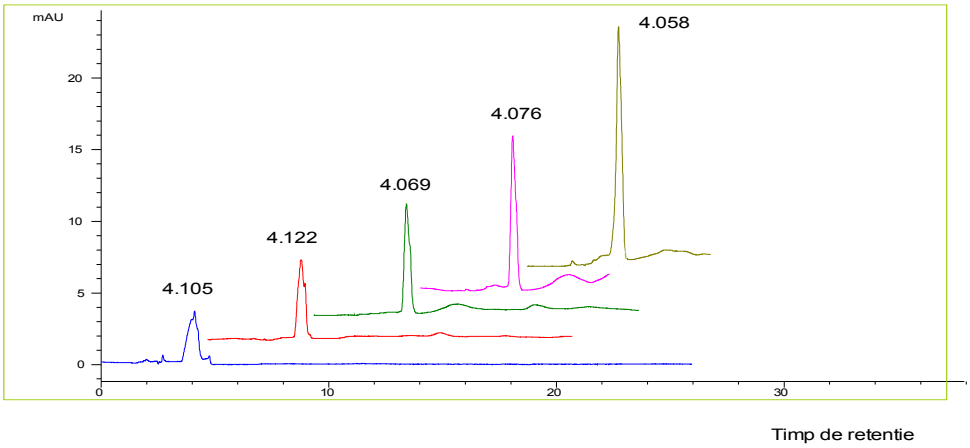

Figure 1. **RP-HPLC superposed chromatograms for standard β-carotene**

β-Carotene was identified in all maize carotenoidic extracts (figures 2-4).

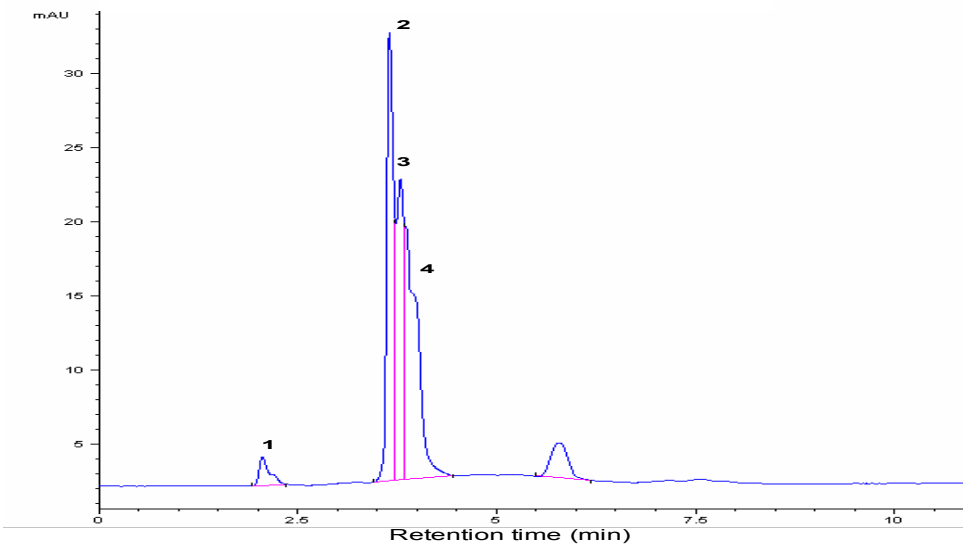

Figure 2. **RP-HPLC chromatograme for *Lovrin 400* hybrid carotenoidic extract**

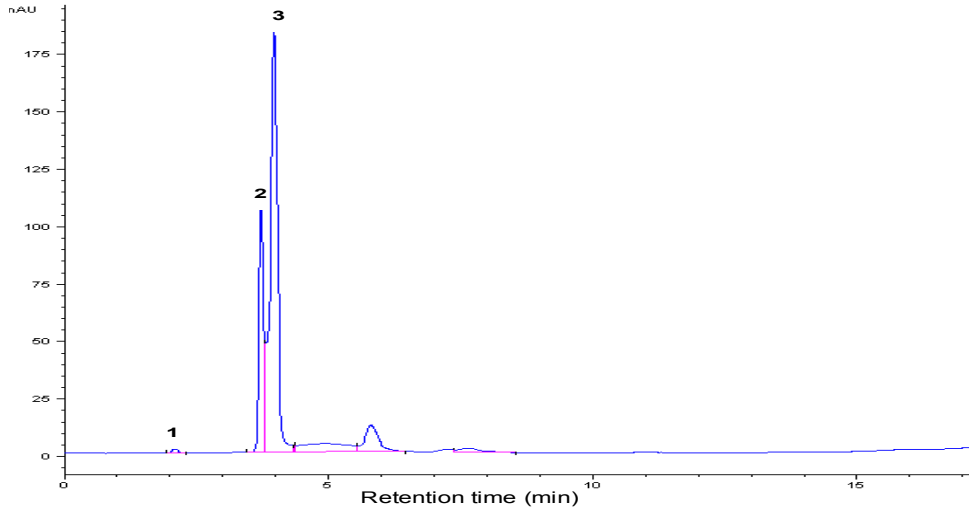

Figure 3. **RP-HPLC chromatograme for *NSSK640* hybrid carotenoidic extract**

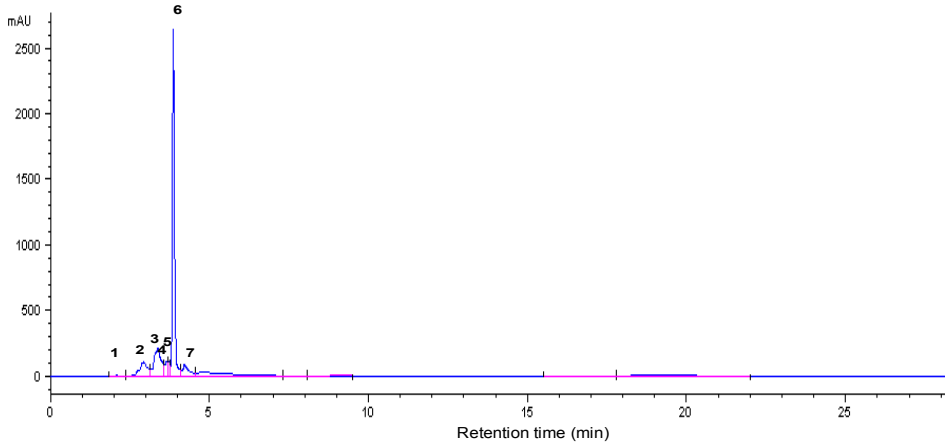

Figure 4. **RP-HPLC chromatograme for *NSSK444* hybrid carotenoidic extract**

The chromatographic peaks area and the β-carotene concentration in the raw material are presented in tables 1-3.

From the tables 1-3 is observed that the all three maize hybrids have a very high β-carotene content, the *NSSK444* hybrid having the best content (670,30 µg/g), followed by the *NSSK640* hybrid (444,60 µg/g) and *Lovrin 400* hybrid (115,50 µg/g).

The obtained values, far greater than some literature data concerning β-carotene content in others maize hybrids (2-18 µg/g) [4,11,12], could be explained by the genetics researches from the last years, towards the obtaining of some new maize hybrids with a high carotenoidic pigments content, especially β-carotene and other provitaminic carotenoids.

Table 1

Chromatographic peaks area and β-carotene concentration in the raw material for *Lovrin 400* hybrid

No. peak	Retention time (min)	Area (Mau min)	A%	Compound identified with standard	Content in the raw material (µg/g)
1	2.047	17.60	3.47	-	-
2	3.644	182.50	36.03	-	-
3	3.777	119.10	23.51	-	-
4	3.841	187.30	36.98	β-carotene	115.50

Table 2

Chromatographic peaks area and β-carotene concentration in the raw material for *NSSK640* hybrid

No. peak	Retention time (min)	Area (mAU min)	A%	Compound identified with standard	Content in the raw material (µg/g)
1	2,083	14,90	0,62	-	-
2	3,695	703,20	29,44	-	-
3	3,955	1670,80	69,94	β-carotene	444,60

Table 3

Chromatographic peaks area and β-carotene concentration in the raw material for *NSSK444* hybrid

No. peak	Retention time (min)	Area (mAU min)	A%	Compound identified with standard	Content in the raw material (µg/g)
1	2.074	117.27	0.61	-	-
2	2.884	2144.18	11.13	-	-
3	3.359	3339.42	17.33	-	-
4	3.644	772.46	4.01	-	-
5	3.726	517.90	2.69	-	-
6	3.844	10996.41	57.08	β-Carotene	670.30
7	4.205	1381.29	7.17	-	-

Also, in this results, the used extraction method have a great importance, because this method determined the complete discolouring of the maize flour (that in the case of other literature methods utilisation doesn't happened). Thus, the maize flour maceration with water and ethanol 96%, before the extraction, allowed the starch hydrolysis and advanced liberation of carotenoids from cells. Superior results concerning β-carotene content in maize was obtained by Egesel et al. [4] for others high β-carotene maize hybrids: 0,7-1,5 mg/g.

CONCLUSIONS

As a result of the achieved researches it could take out the following conclusions:

- HPLC method is very adequate for β-carotene determination in maize flour because is: sensitive, selective, fast, reproducible and reliable.

- The all analysed maize hybrids are very rich iņ β-carotene, the obtained values being far greater than that from some literature data for others maize hybrids. From this point of view, these maize hybrids have a very high nutritive value.

- *NSSK444* hybrid presents the beşt β-carotene content (670,30 μg/g), followed by the *NSSK640* hybrid (444,60 μg/g) and *Lovrin 400* hybrid (115,50 μg/g).

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